

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of:

Kennerknecht, *et al.*

(Continuation of Appl. No. 09/471,803)

Appl. No.: to be assigned

Filed: herewith

For: **Nucleotide Sequences Coding for
the Export of Branched-Chain
Amino Acids, Process for the
Isolation Thereof and Use Thereof**

Art Unit: 1645
(1645 in parent case)

Examiner: to be assigned
(J. Graser in parent case)

Atty. Dkt. 7601/80525

Amendment to Comply with Sequence Listing Rules

Commissioner of Patents
U.S. Patent and Trademark Office
2011 South Clark Place
Customer Window, MS Patent Application
Crystal Plaza Two, Lobby, Room 1B03
Arlington, VA 22202

Sir:

Applicants are submitting the present Amendment in order to comply with Sequence Listing Rules. All page numbers recited below refer to the certified English translation of the application submitted herewith.

Amendments

Please delete the Sequence Listing originally filed with the application and appearing as pages 30-36 of the specification. Please enter the substitute Sequence Listing enclosed herewith on separate pages following the claims and abstract of the application.

On page 26 of the specification, please amend lines 16-19 to read as follows:

brnE, brnF, -forward:

5'-(AGC GCT GTC TGC TTA AGC CTT TTC)-3' (SEQ ID NO: 7)

brnE, brnF, -reverse:

5'-(GCG CGA TCA ATG GAA TCT AGC TTC)-3' (SEQ ID NO: 8)

On page 27, line 27, to page 28, line 2 of the specification, please amend to read as follows:

Universal primer:

5'-GTA AAA CGA CGG CCA GT-3' (SEQ ID NO: 9)

Reverse primer:

5'-GGA AAC AGC TAT GAC CAT G-3' (SEQ ID NO: 10)

On page 24, line 13, to page 25, line 12 of the specification, please amend the text to read as follows:

In order to clone the insertion site located downstream from transposon Tn5531 of the mutant described in Example 1.1, the chromosomal DNA of this mutant strain was first isolated as described in Schwarzer et al. (Bio/Technology (1990) 9: 84-87) and 400 ng thereof were cut with the restriction endonuclease EcoRI. The complete restriction batch was ligated into the vector pUC 18 (Norander et al., Gene (1983) 26: 101-106), likewise linearised with EcoRI, from Roche Diagnostics (Mannheim, Germany). The *E. coli* strain DH5amcr (Grant et al., Proceedings of the National Academy of Sciences of the United States of America (1990) 87: 4645-4649) was transformed with the entire ligation batch by means of electroporation (Dower et al., Nucleic Acid Research (1988) 16: 6127-6145). Transformants in which the insertion sites of transposon Tn5531 were present in cloned form on the vector pUC 18 were identified by means of the carbenicillin and kanamycin resistance on LB agar plates containing 50 µg/mL of carbenicillin and 25 µg/mL of kanamycin. The plasmids were prepared from three of the transformants and the size of the cloned inserts determined by restriction analysis. The nucleotide sequence of the insertion site on one of the plasmids having an insert of a size of approx. 7.2 kb was determined using the dideoxy chain termination method of

Sanger *et al.* (Proceedings of the National Academy of Sciences of the United States of America (1977) 74: 5463-5467). To this end, 1.3 kb of the insert were sequenced starting from the following oligonucleotide primer:

5'-CGG GTC TAC ACC GCT AGC CCA GG-3' (SEQ ID NO: 11).

On page 25 of the specification, please amend lines 13-25 to read as follows:

In order to identify the insertion site located upstream from the transposon, the chromosomal DNA of the mutants was cut with the restriction endonuclease PstI and ligated into vector pUC 18 which had been linearised with PstI. The remainder of the cloning operation was performed as described above. The nucleotide sequence of the insertion site on one of the plasmids having an insert of a size of approx. 4.8 kb was determined using the dideoxy chain termination method of Sanger *et al.* (Proceedings of the National Academy of Sciences of the United States of America (1977) 74: 5463-5467). To this end, 1.6 kb of the insert were sequenced starting from the following oligonucleotide primer: 5'-CGG TGC CTT ATC CAT TCA GG-3' (SEQ ID NO: 12).